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(71) Applicant: **GAMIDA SENSE DIAGNOSTICS LTD.**  
**77610 Ashdod (IL)**

(72) Inventors:

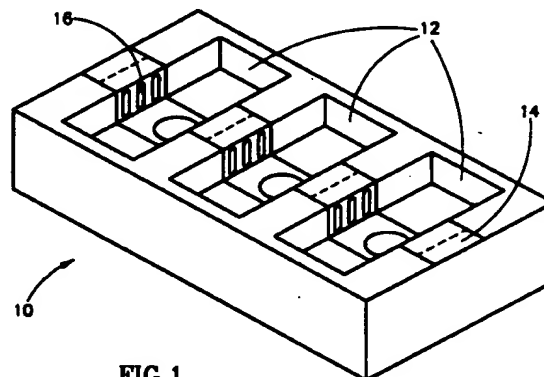
• **Alajem, Sara**  
**76875 Kfar (IL)**

• **Ritterband, Menahem**  
**74046 Nes Zlona (IL)**  
• **Reinhartz, Avraham**  
**76450 Rehovot (IL)**

(74) Representative: **Modiano, Guido, Dr.-Ing. et al**  
**Modiano, Josef, Pisanty & Staub,**  
**Baaderstrasse 3**  
**80469 München (DE)**

**(54) Apparatus and method for detection of analytes in a sample**

(57) Apparatus for detection one or more analyte in a fluid sample. The apparatus includes one or more fluid including the fluid sample to be tested for the analyte(s) and one or more vessels for holding the fluid(s). The vessel includes a base at a first end, side walls extending generally perpendicularly from the base to a second end, an opening in the vessel defined by the side walls and the second end and a central axis extending from the center of the base to the center of vessel opening. The apparatus further includes a single matrix sheet including at least one capture site for capturing a specific analyte which is inserted in vessel and spaced from the central axis opposite the side walls and at least one apparatus for detection of a captured specific analyte.



**FIG. 1**

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## Description

### FIELD AND BACKGROUND OF THE INVENTION

The invention relates to a method and apparatus for detection of analytes in a fluid sample.

The use of test devices for detection of analytes is well known in the art. For example, U.S. Patent 4,803,170 to Stanton et al. describes a device for detecting an analyte in a fluid sample including a reaction vessel, and non-overlapping reaction and detection surfaces disposed irreversibly in the vessel. The device described by Stanton et al, however, detects only single analyte in a fluid sample.

### SUMMARY OF THE INVENTION

The present invention provides an improved apparatus for detection of analytes in a fluid sample. There is thus provided apparatus for detection of at least one analyte in a fluid sample including at least one fluid including the fluid sample to be tested for the at least one analyte, at least one vessel for holding the at least one fluid including, a base at a first end, side walls extending generally perpendicularly from the base to a second end, an opening in the vessel defined by the side walls and the second end and a central axis extending from the center of the base to the center of the vessel opening, a single matrix sheet including at least one capture site for capturing a specific analyte, inserted in the vessel and spaced from the central axis opposite the side walls, and at least one apparatus for detection of a captured specific analyte. According to a preferred embodiment of the invention the single sheet matrix comprises at least one member.

According to another preferred embodiment of the invention the single sheet matrix comprises a plurality of members. According to yet another preferred embodiment of the invention at least two of the plurality of members at least partially overlap.

According to still another preferred embodiment of the invention the at least one capture site includes at least one capture reagent irreversibly fixed to the at least one capture site. According to a further preferred embodiment of the invention at least a portion of the single matrix sheet is spaced at least 0.001 mm from the base and side walls.

According to yet a further preferred embodiment of the invention the at least one capture site including a plurality of capture sites. According to still a further preferred embodiment of the invention the plurality of capture sites include at least two, at least partially overlapping capture sites.

According to another preferred embodiment of the invention the apparatus for detection analytes in a fluid sample also includes a pipette probe which may be inserted through the opening into the vessel along the central axis to introduce the at least one fluid into the

vessel and to remove the at least one fluid from the vessel.

According to yet another preferred embodiment of the invention the at least one apparatus for detection includes a detection reagent which binds to a captured analyte, a fluid containing the detection reagent, and signaling apparatus for signaling the presence of the specific analyte. According to still another preferred embodiment of the invention the signaling apparatus includes a sensible signal bound to the detection reagent. According to a further preferred embodiment of the invention the sensible signal is a color signal.

According to still a further preferred embodiment of the invention the signaling apparatus includes, a developable signal bound to the detection reagent, a developing reagent, and a fluid containing the developing reagent. According to yet a further preferred embodiment of the invention the developable signal is a color signal.

According to another preferred embodiment of the invention the developable signal is retained in the capture site. According to yet another preferred embodiment of the invention the developable signal is contained in the fluid containing the developing reagent.

According to a further preferred embodiment of the invention the capture reagent comprises an antibody.

According to yet a further preferred embodiment of the invention the capture reagent comprises an antigen.

According to still a further preferred embodiment of the invention the capture reagent comprises a DNA sequence.

According to another preferred embodiment of the invention the capture reagent comprises an RNA sequence.

According to yet another preferred embodiment of the invention the detection reagent comprises an antibody.

According to still another preferred embodiment of the invention the detection reagent comprises an antigen.

According to a further preferred embodiment of the invention the detection reagent comprises a DNA sequence.

According to still a further preferred embodiment of the invention the detection reagent comprises an RNA sequence.

According to yet another preferred embodiment of the invention the vessel comprises a generally light transmissive vessel.

According to a further preferred embodiment of the invention the single matrix sheet is removably inserted in the at least one vessel. According to yet a further preferred embodiment of the invention the single matrix sheet is spaced between 0.5mm and 10mm from the central axis.

According to still a further preferred embodiment of the invention the single matrix sheet is inserted into a single vessel.

According to another preferred embodiment of the invention the single matrix sheet is inserted into a plurality of vessels.

According to yet another preferred embodiment of the invention the single matrix sheet includes a window opposite the base for light transmission through the light transmissive vessel along the central axis.

There is also provided by the present invention a method for detection of at least one analyte in a fluid sample comprising the steps of, providing a vessel for holding a fluid sample, providing a single matrix sheet inserted in the vessel and spaced from the central axis including a capture site wherein at least one capture for capturing a specific analyte is immobilized to the capture site and, introducing a volume of a fluid sample to be tested for at least one analyte, incubating the fluid sample in the vessel for a predetermined period of time, removing the fluid sample, introducing a volume of a fluid containing a detection reagent which binds the analyte and is conjugated to a color producing enzyme, incubating the fluid containing the detection reagent for a predetermined period of time, removing the fluid containing the detection reagent, introducing a fluid containing a developing reagent wherein a reaction between the developing reagent and the detection reagent produces a visible color reaction in the capture site which is a function of analyte concentration.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be understood and appreciated more fully from the following detailed description taken in conjunction with the drawings in which:

FIG. 1 a pictorial illustration of a preferred embodiment of an apparatus for detection of analytes a fluid sample constructed and operative in accordance with the present invention;

FIG. 2 is a pictorial representation of a portion of a the apparatus of Figure 1;

FIG. 3 is a side sectional illustration of another preferred embodiment of the apparatus of Figure 1; and

FIG. 4 is a side sectional illustration of yet another preferred embodiment of the apparatus of Figure 1;

FIG. 5 is yet another embodiment.

#### DESCRIPTION OF THE PREFERRED EMBODIMENTS

Reference is now made to Figures 1 - 4 which illustrate apparatus 10 for detection of at least one analyte in a fluid sample constructed and operative in accordance with preferred embodiments of the present invention.

Apparatus 10 includes a single vessel 12 (best seen in Figures 3 and 4) or a plurality of vessels (12) (best seen in Figure 1). A single matrix sheet (14) is disposed in the vessels 12. The single sheet matrix 14 is typically constructed from one or more members each member being fabricated from binding materials such as nitrocellulose, cellulose, nylon or glass fibers.

The single sheet matrix 14 may include members fabricated from one or more materials and includes at least one capture site 16. The single sheet matrix 14 may be fabricated with one or more members overlapping each other and with one or more capture sites 16. The capture sites 16 may also either be separate or at least partially overlap. The matrix sheet 14 is typically planar but is folded along lines 18 (best seen in Figure 2) to permit insertion of the matrix sheet 14 in the vessels 12. The matrix sheet 14 is typically disposed in vessels 12 spaced between 0.5mm and 10.0mm, preferably between 2.9mm and 3.1mm from the central axis 20 and between 0.001mm and 2.9mm from a base 22 and side walls 24 of vessels 12. The matrix 14 is preferably removably disposed in the vessels 12, although it may be fixed entirely or partially to the base and walls. The vessels 12 are typically fabricated from polystyrene, and the base 22 and side walls 24 are typically light transmissive.

The matrix sheet 14 in the area of the base 22 may be formed with a window 26 to allow light to pass through the vessel 12 along the central axis 20. The matrix sheet 14 in the area of the opening in the vessel which defined by the side walls and the second end, may be formed with a gripping area 28 to allow removing the matrix sheet 14 from vessel 12 (best seen in Figure 5). The matrix sheet 14 in the area of the gripping area 28 may be connected each other in a tearing line 30 to allow separation between the connected matrix sheet 14 during or after pulling the matrix sheets 14 out of vessels 12 within apparatus 10 (best seen in Figure 2). In the use of apparatus 10, a fluid sample 20  $\mu$ l to 1000  $\mu$ l which may contain one or more analytes to be detected is disposed in the vessels 12, using a mechanically or manually operated pipette inserted in the vessel along the central axis 20. The sheet matrix 14 including one or more capture sites 16 is disposed in the vessels 12.

Before being inserted in the vessels 12 at least one capture reagent is irreversibly fixed by chemical or physical binding procedures to one or more capture sites 16. The capture reagent is typically an antigen, antibody, RNA or DNA sequence which binds the analyte. The fluid sample is allowed to incubate in the vessel 12 for between 1 min and 24 hours and is then removed by pipette. A volume of fluid of between 20  $\mu$ l to 1ml containing a detection reagent which binds the analyte and which is also bound to a sensible signal is then allowed to incubate in the vessel 12 for between 1 minute and 24 hours.

The detection reagent is typically an antibody, antigen, DNA or RNA sequence. The sensible signal is typ-

ically a signal generating compound such as enzymes, colored particles or light emitting substances and is bound to the detection reagent by chemical or physical reactions. The sensible signal may itself be detectable itself as a color change in the capture area as with colored particles or light. The sensible signal, however, may also be a developable signal, typically an enzymatic reaction where a substrate converts a chromogen into a visually detectable signal. If the signal is a developable signal, then fluid containing the detection reagent may be replaced with a 20µl to 1 ml of a fluid containing a development reagent such as o-phenylene-diamine.2HCL.

The development reagent produces a sensible reaction such as a color reaction either in the fluid or the at the capture site. The sensible signal may be detected in the vessel by visual observation of the fluid in the vessel or capture site or by using instrumentation for observation of either the fluid or the capture site like spectrophotometer reading for the fluid and scanner reading for the capture site. The sensible signal may also be detected by observation of the sheet matrix after removal of the matrix from the vessels. Reference is now made to the following examples which, together with Figures 1 - 4 illustrate the invention.

#### Example 1

##### Detection of Human Chorionic Gonadotrophin (HCG). Preparation of the sheet matrix.

A nitrocellulose (NC) membrane, pore size 0.45µm (BioRad, Rockville Center, NY, No. 162-0115) is cut into primary sheets 8cm wide x 8cm long and placed into 5ml solution containing the following capture reagent, 100µg/ml purified IgG1 monoclonal antibody anti-alpha-HCG (Accurate Chemical & Scientific Co., Westbury, NY) in phosphate-buffered saline + 0.01% sodium azide (PBSA) at room temperature for 1 hour. The non-specific binding sites are blocked by transferring the membrane to a 250ml solution containing 10mg/ml BSA and 0.05% Tween-20 (both from Sigma, St. Louis, MO, USA), in PBSA and placed for additional incubation for one hour. The membrane is then rinsed briefly with distilled water and allowed to dry at room temperature.

The primary sheet is cut into pieces of 0.4cm wide / 2cm long to form the sheet matrix comprising the capture reagent. The matrix is inserted into wells of a commercially available microtitration plate (Nunc, Roskilde, Denmark).

#### Assay Procedure

PBS solution containing the analyte at concentrations of 0, 25, 100, 250 mIU/ml of HCG are tested in replicates in two identical microtitration plates inserted with matrix sheets with the same assay procedure. The assay procedure is performed manually (plate "A"), or using an automated procedure (plate "B").

The assay procedure:

- 1) addition of 100µl sample to a well and incubation for 20 min at room temperature (RT).
- 2) washing of wells by adding 250µl washing solution (0.3% Tween-20 in PBSA) twice.
- 3) addition of 100µl of a solution containing enzyme labeled monoclonal antibody, anti-beta-HCG Horseradish Peroxidase conjugate (1:1000) (Biogenesis Inc., Sandown, NH.), and incubation for 20 min at RT.
- 4) washing as in step 2 four times.
- 5) addition of 200µl of o-phenylene-diamine .2HCl (OPD) in substrate solution (phosphate-citrate buffer containing hydrogen peroxide), incubation for 20 min at RT.
- 6) addition of 50µl of stop solution containing 2 N sulfuric acid 7) spectrophotometric reading of plates at 492nm, for detection of HCG when present in samples, both in manually operated or automated procedures.

#### Example 2

##### Detection of HCG and test validity control.

Preparation of the sheet matrix:

First member:

A nitrocellulose membrane, pore size 0.45µm is cut into primary sheets of 8cm x 8cm and placed into a 5ml solution containing 100µg/ml purified IgG1 monoclonal antibody anti-alpha-HCG in PBSA at room temperature for 1 hour. The non-specific binding sites are blocked by transferring the membrane to a 250ml solution containing 10mg/ml BSA and 0.05% Tween-20 in PBSA and placed for additional incubation for one hour. The membrane is then rinsed briefly with distilled water and allowed to dry at room temperature. The primary sheet is further cut into pieces 0.4cm wide x 2cm long to form the first member of the sheet matrix. The first member of the sheet matrix is then placed at the bottom of a microtitration well.

Second member:

A molded ring-shaped cylinder having a thin wall (0.1mm) and a height of 0.2cm, consisting of high-impact-polystyrene (HIPS) as a matrix sheet, is placed into a 5ml solution containing 25µg/ml purified IgG monoclonal antibody anti-human-hemoglobin in PBSA, at room temperature for 1 hour. The non-specific binding sites are blocked by transferring the HIPS cylinder to a

250ml solution containing 10mg/ml BSA and 0.05% Tween-20 in PBSA and placed for an additional incubation of one hour. The HIPS is then rinsed briefly with distilled water and allowed to dry at room temperature. The second member of the matrix sheet is then inserted into the same microtitration well, above the first member, so that both matrix surfaces are in contact.

#### Assay Procedure:

Diluted serum (1/100) in PBS, with a final concentration of 0, 25, 100, 250 mIU/ml HCG is tested in two identical microtitration plates (A, B). In each plate samples without diluted serum are added as validation controls.

#### The assay procedure:

- 1) addition of 150µl sample to a well and incubation for 20 min at RT.
- 2) washing of wells by adding 250µl washing solution (0.3% Tween-20 in PBSA) twice.
- 3) addition of 150µl of a solution containing a mixture of enzyme-labelled antibodies, anti-beta-HCG β-galactosidase conjugate, and anti-human-hemoglobin alkaline phosphatase conjugate. Incubation for 20 min at room temperature.
- 4) washing as in step 2, four times.

#### Signal development plate A:

- 5) addition of 250µl of oNPG substrate solution, o-nitrophenyl-β-D-galactoside in 100mM Tris HCl pH 7.5, incubation for 30 min at RT.
- 6) Immediately, spectrophotometric reading of the plate at 420nm, for detection of HCG when present in the sample.
- 7) washing as in step 2.
- 8) addition of 200 µl of BCIP-NBT substrate solution, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium in 100mM Tris HCl pH 9.5, 5mM MgCl<sub>2</sub> buffer, incubation for 10 min.
- 9) visual reading of blue/purple precipitated color is retained in unit B, indicates a successful test run.

#### Signal development plate B:

- 5) addition of 100µl of oNPG substrate solution, o-nitrophenyl-β-D-galactoside in 100mM Tris HCL pH 7.5, incubation for 30 min at RT.

6) addition of 100µl of PMP substrate solution, phenolphthalein monophosphate in 100mM sodium carbonate pH 9.9, 10mM MgCl<sub>2</sub> buffer, incubation for 30 min at RT.

7) addition of 50µl 0.25N sodium hydroxide.

8) The two developed signals are read spectrophotometrically at 420nm and 540nm.

The OD at 420 nm which is obtained for each sample is proportional to the HCG concentrations in the samples. The OD at 540nm which is obtained for the different samples indicates the validity of the assay for each sample.

#### Example 3

##### Detection of specific DNA by hybridization.

##### Preparation of the sheet matrix:

A nitrocellulose membrane, pore size 0.45µm, is cut in a pattern similar to that shown in Figure 2. Three different DNA sequences are immobilized to the matrix sheet at three different capture sites; pBR322 (I); Lambda DNA/Hind III (II); ox 174,RF DNA (III) (Promega, Madison, USA). The DNA sequences, each as a 10pg/µl solution in 10xSSC, are spotted (1µl) at intervals of 0.5cm onto the sheet matrix. The DNAs are fixed to the membrane by baking at 80°C for 2 hours. The matrix sheet are inserted to microtiter wells.

##### Sample labeling:

pBR322, Lambda DNA/Hind III, ox 174, RF and a mixture of the three DNAs are labeled by the random primer DNA labeling method with digoxigenin dUTP according to the DIG DNA Labeling Kit protocol Boehringer Biochemica, Mannheim, Germany. The four samples of labeled DNA's are diluted in the hybridization solution according to DIG Nucleic-Acid detection Kit at final concentration of 100ng/ml. The DNAs are heat denaturated and kept at 4°C.

##### Hybridization in Formamide solution:

The assay procedure is performed according to the DIG Nucleic-Acid detection Kit.

1) Addition of 150µl prehybridization solution containing 200µg/ml salmon sperm DNA (Sigma, St. Louis, USA) to four different wells A,B,C and D, and incubation at 42°C for 1 hour.

2) discarding of the prehybridization solution and addition of 150µl of the hybridization solution containing the labeled DNA pBR 322 to well A; Lambda DNA/Hind III to well B; ox 174 rf to well C and the

DNA mixture to well D. Incubation for 1 hour at 42°C.

3) Washing in 250µl of wash solution 1 containing 2xSSC, 0.1 %SDS, seven times at RT.

4) Washing in 250µl of wash solution 2 containing 0.1xSSC, 0.1%SDS, seven times at RT.

5) Washing in 250µl of wash solution 3 containing 100mM Tris HCL, 150 mM sodium chloride at pH 7.5 at RT.

6) addition of 150µl blocking solution and incubation for 30 min at RT.

7) Addition of anti-digoxigenin/alkaline-phosphatase conjugate and incubation for 30 min at RT.

8) Washing as in step 5, four times.

9) Addition of 200µl of color substrate solution and incubation for 15 min at RT.

10) Washing as in step 8.

11) Removing the matrix sheet from the microtitration wells by grasping the projection (designed for facilitating handling of the matrix) and visually reading the precipitated color.

A visible spot will appear on the capture sites whenever the sample contains homologous DNA.

#### Example 4

##### Detection of specific antibody

Preparation of the sheet matrix:

The sheet matrix is prepared as described in example 1 except that the capture reagent which is immobilized to the matrix is a specific antigen.

Assay procedure:

A. Sample containing the antibody analyte is incubated with the capture reagent antigen, which is first immobilized on the matrix. The unbound antibody is washed off. The captured antibody is detected by adding to the well solution containing antigen conjugated to alkaline phosphatase (AP), specifically recognizing the antibody. The uncombined conjugate is washed off and a solution containing OPD is added. Spectrophotometric reading of the well at 492nm, for detection of the presence of the antibody in the sample and its level.

B. The same experiment is repeated, using a gold-labeled conjugate in place of the AP/antigen conjugate. Development of color on the capture sites indicates the presence of antibody in the sample.

#### Example 5

##### Detection of HCG, with a control test for validation, employing a single-member sheet matrix.

The experiment described in example 2 is repeated using overlapping capture sites located on a single-member matrix sheet. Preparation of the matrix sheet:

The NC sheet membrane is placed in a solution containing both capture reagents immobilized and inserted into the well.

Assay procedure:

Assay procedure is repeated as described for "plate B" example 2. The OD at 420 nm which is obtained is proportional to the HCG concentration in the samples. The OD at 540nm which is obtained for the different samples indicates the validity of the assay for each sample.

#### Example 6

##### Detection of HCG with a control test for validation

Preparation of the matrix sheet:

The first and the second members of the matrix sheet are prepared as described in example 2. The first member, 0.4 cm wide × 2 cm long, and the second member, 0.2 cm wide × 2 cm long, are glued side by side at a distance of 0.1 cm on a third member of the matrix sheet, a nylon net, 0.7 cm wide × 2 cm long.

Assay procedure:

As in example 2.

#### Example 7

Example 1 is repeated except that the matrix sheet is inserted into a flat-bottom tube with a diameter of 0.64 cm and a height of 4 cm. The assay procedure is performed manually as for "plate A" in example 1.

#### Example 8

Example 3 is repeated except that the matrix sheet is without the window (26) best shown in Figure 3.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and other applications of the invention may be made. Where technical features mentioned in any claim are followed

by reference signs, those reference signs have been included for the sole purpose of increasing the intelligibility of the claims and accordingly such reference signs do not have any limiting effect on the scope of each element identified by way of example by such reference signs.

#### Claims

1. Apparatus for detection of at least one analyte in a fluid sample comprising: at least one fluid including the fluid sample to be tested for the at least one analyte; at least one vessel for holding the at least one fluid including a base at a first end, side walls extending generally perpendicularly from the base to a second end, an opening in the vessel defined by the side walls and the second end and a central axis extending from the center of the base to the center of vessel opening; a single matrix sheet including at least one capture site for capturing a specific analyte, inserted in vessel and spaced from the central axis opposite the side walls; and at least one apparatus for detection of a captured specific analyte. 10
2. Apparatus according to claim 1 wherein the vessel is at least one well of a microtitration plate. 15
3. Apparatus according to claim 1 wherein the single sheet matrix comprises at least one member. 20
4. Apparatus according to claim 2 wherein the single sheet matrix comprises a plurality of members. 25
5. Apparatus according to claim 3 wherein at least two of the plurality of members at least partially overlap. 30
6. Apparatus according to claim 1 wherein the at least one capture site includes at least one capture reagent irreversibly fixed to the at least one capture site. 35
7. Apparatus according to claim 1 an additionally including at least a portion of the single matrix sheet spaced at least 0.001 mm from the base and side walls. 40
8. Apparatus according to claim 1 an additionally including a plurality of capture sites. 45
9. Apparatus according to claim 7 wherein the plurality of capture sites include at least two, at least partially overlapping capture sites. 50
10. Apparatus according to claim 1 and additionally including a pipette probe which may be inserted through the opening into the vessel along the central axis to introduce the at least one fluid into the vessel and to remove the at least one fluid from the vessel. 55
11. Apparatus according to claim 1 wherein the at least one apparatus for detection includes: a detection reagent which binds to a captured analyte; a fluid containing the detection reagent; and signaling apparatus for signaling the presence of the specific analyte.
12. Apparatus according to claim 10 wherein the signaling apparatus includes a sensible signal bound to the detection reagent.
13. Apparatus according to claim 11 wherein the sensible signal is a color signal.
14. Apparatus according to claim 10 wherein the signaling apparatus includes: a developable signal bound to the detection reagent; a developing reagent; and a fluid containing the developing reagent.
15. Apparatus according to claim 13 wherein the developable signal is a color signal.
16. Apparatus according to claim 14 wherein the developable signal is retained in the capture site.
17. Apparatus according to claim 14 wherein the developable signal is contained in the fluid containing the developing reagent.
18. Apparatus according to claim 5 wherein the capture reagent comprises an antibody.
19. Apparatus according to claim 5 wherein the capture reagent comprises an antigen.
20. Apparatus according to claim 5 wherein the capture reagent comprises a DNA sequence.
21. Apparatus according to claim 5 wherein the capture reagent comprises an RNA sequence.
22. Apparatus according to claim 10 wherein the detection reagent comprises an antibody.
23. Apparatus according to claim 10 wherein the detection reagent comprises an antigen.
24. Apparatus according to claim 10 wherein the detection reagent comprises a DNA sequence.
25. Apparatus according to claim 10 wherein the detection reagent comprises an RNA sequence.
26. Apparatus according to claim 1 wherein the vessel comprises a generally light transmissive vessel.

27. Apparatus according to claim 1 wherein the single matrix sheet is removably inserted in the at least one vessel.
28. Apparatus according to claim 1 wherein the single matrix sheet is spaced between 0.5mm and 10.mm from the central axis. 5
29. Apparatus according to claim 1 wherein the single matrix sheet is inserted into a single vessel. 10
30. Apparatus according to claim 1 wherein the single matrix sheet is inserted into a plurality of vessels.
31. Apparatus according to claim 23 wherein the single matrix sheet includes a window opposite the base for light transmission through the light transmissive vessel along the central axis. 15
32. A method for detection of at least one analyte in a fluid sample comprising the steps of: providing a vessel for holding a fluid sample; providing a single matrix sheet inserted in vessel and spaced from the central axis including a capture site wherein at least one capture for capturing a specific analyte is immobilized to the capture site and; introducing volume of a fluid sample to be tested for at least one analyte; incubating the fluid sample in the vessel for a predetermined period of time; removing the fluid sample; introducing a volume of a fluid containing a detection reagent which binds the analyte and is conjugated to a color producing enzyme; incubating the fluid containing the detection reagent for a predetermined period of time; removing the fluid containing the detection reagent; introducing a fluid containing a developing reagent wherein a reaction between the developing reagent and the detection reagent produces a visible color reaction in the capture site which is a function of analyte concentration. 20 25 30 35 40

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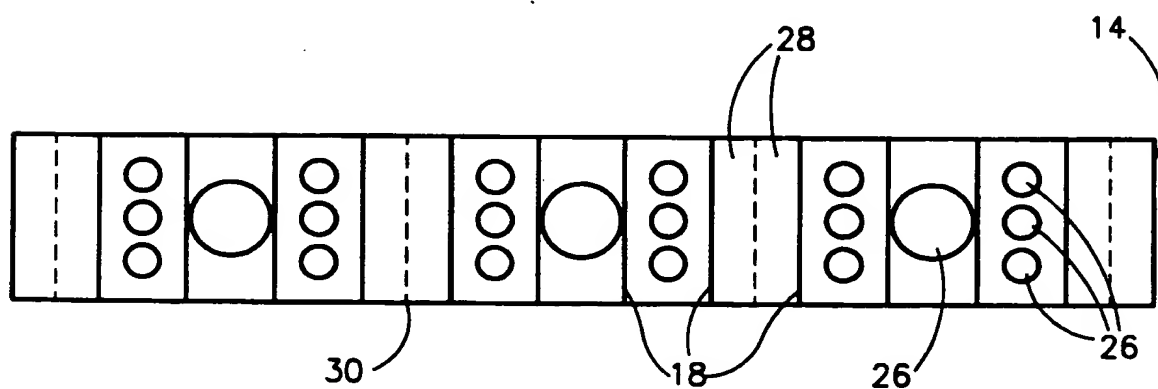
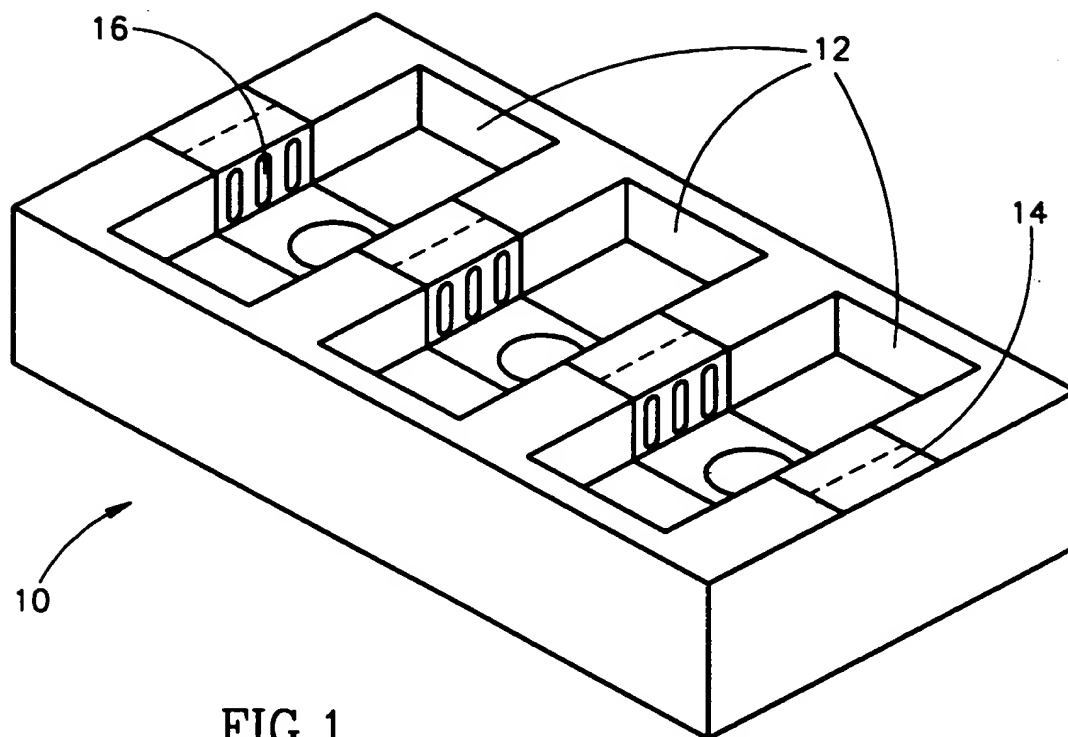


FIG. 2

FIG.3

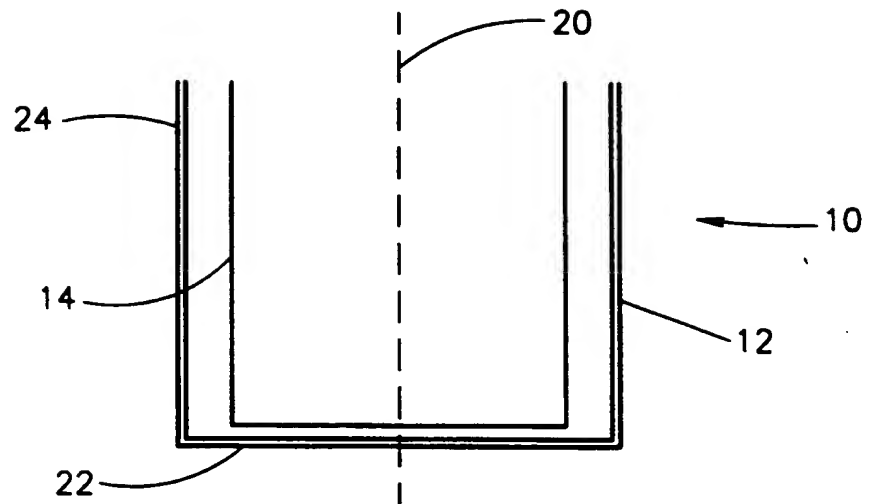


FIG.4

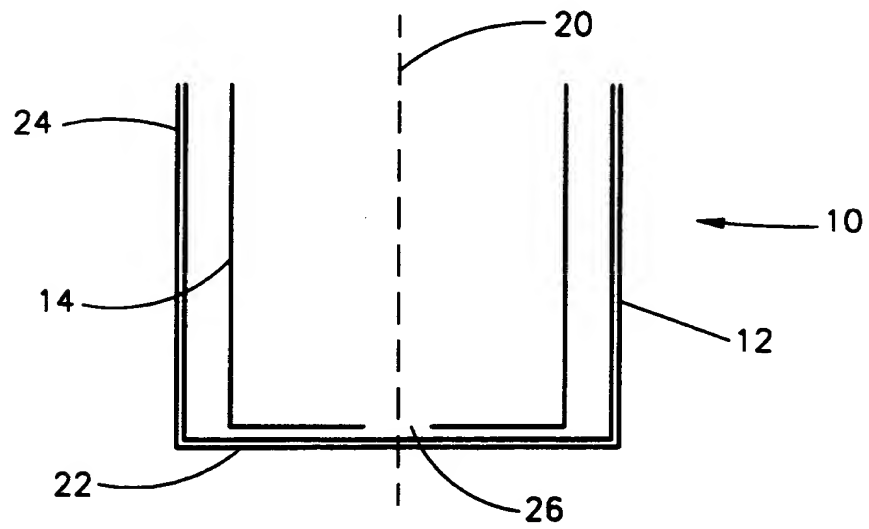


FIG.5

